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EFFECTS OF PRE-SOWING SOIL TREATMENTS ON ROOT COLONIZATION OF 1-0 PONDEROSA AND LODGEPOLE PINE SEEDLINGS BY POTENTIALLY-PATHOGENIC FUNGI USDA FOREST SERVICE LUCKY PEAK NURSERY BOISE, IDAHO

R.L. James, Plant Pathologist

ABSTRACT

Healthy-appearing 1-0 ponderosa and lodgepole pine seedlings were assayed for root infection and colonization by potentially-pathogenic fungi following pre-sowing soil treatments which included methyl bromide/chloropicrin (MBC) fumigation, fallowing with or without periodic cultivation, and amending soil with mushroom composts or undecomposed sawdust. Levels of root colonization by *Fusarium* spp. on both conifer species were lowest in MBC-treated fields. Fallowing, particularly without cultivation, was nearly as beneficial in reducing *Fusarium* root colonization for lodgepole pine seedlings. These results correlated well with 2-0 seedling densities in treated fields. *Fusarium oxysporum* was the most common colonizer of roots of healthy seedlings as well as the most important cause of seedling disease. Continued efforts are underway to develop satisfactory alternatives to MBC fumigation at the Lucky Peak Nursery.

INTRODUCTION

Pre-plant soil fumigation in forest nurseries is used to control soil-borne pathogens and weeds (James 1989). Traditionally, methyl-bromide chloropicrin (MBC) (67% and 33%, respectively) has been the fumigant of choice in most forest nurseries (Fraedrich 1993; James 1989). However, recent concerns about the potential of methyl bromide to deplete stratospheric ozone (World Meteorological Association 1995), has restricted use of this fumigant. The U.S. Clean Air Act specifies that methyl bromide will no longer be manufactured or used in the United States after January 2005 (NAPIAP 1993). As a result, efforts have been underway for the past several years to develop viable alternatives to soil fumigation in general and methyl bromide in particular in western forest nurseries (James et al. 1994b; Stone et al. 1995, 1997).

A recent trial at the USDA Forest Service Lucky Peak Nursery near Boise, Idaho was designed to compare four alternative pre-plant soil treatments with standard MBC fumigation. Effects of these treatments on

United States Department of Agriculture | Forest Service | Northern Region | 200 East Broadway P.O. Box 7669 Missoula, MT 59807

seedling disease, density, and biomass production were previously reported (Stone et al. 1997). The current report details effects of these treatments on root colonization of healthy-appearing 1-0 ponderosa (*Pinus ponderosa* Laws.) and lodgepole (*Pinus contorta* Dougl.) pine seedlings by potentially pathogenic fungi. The assayed fungi are those that are most commonly associated with young seedling diseases at the nursery.

MATERIALS AND METHODS

Two fields at the Lucky Peak Nursery were evaluated with five pre-sowing treatments during year 1 (Stone et al. 1997). Each treatment was replicated 5 times in a complete randomized block design in each field. The first field was sown with ponderosa pine and the second with lodgepole pine. Treatment blocks measured 15.2 x 1.6 m (50 x 6 ft.), with the exception of fumigated blocks that were 15.2 x 3.7 m (50 x 12 ft.). The five treatments were: (A): bare fallow with periodic cultivation to turn the soil and keep weed populations low, (B): bare fallow without cultivation, (C): mushroom compost amendment applied topically and cultivated into the soil, (D): undecomposed sawdust containing supplemental nitrogen applied topically and cultivated into the soil, and (E) standard operational MBC soil fumigation (392kg/ha: 350 lbs./acre). Beds were formed and sown with the appropriate conifer species during the spring of year 2. Standard operational sowing procedures were used.

At the end of the first growing season (October – year 2), seedlings were examined for density and disease. Twenty healthy-appearing ponderosa and lodgepole pine seedlings were randomly selected from each treatment, carefully extracted, and transported to the laboratory for analysis of root colonization.

Seedling roots were washed thoroughly under running tap water to remove adhering soil particles. They were then aseptically dissected into pieces about 5 mm in length. Ten root pieces per seedling were randomly selected, surface sterilized in 0.525% aqueous sodium hypochlorite, rinsed in sterile water, and placed on a selective agar medium for *Fusarium* and closely-related fungi (Komada 1975). Plates with roots were incubated under diurnal cycles of cool, fluorescent light at about 24^{o}C for 7-10 days. Fungi emerging from root pieces were identified to genus. Selected isolates of *Fusarium* and *Cylindrocarpon* were single-spored and transferred to carnation leaf agar (Fisher et al. 1982) and potato dextrose agar (PDA) for identification. *Fusarium* species were identified using the taxonomy of Nelson et al. (1983) and *Cylindrocarpon* species were identified using descriptions of Booth (1966).

Root infection was determined based on whether or not a particular fungus infected any of the sampled seedling roots; root colonization was calculated as the percentage of root pieces (10 per seedling) that were colonized by the appropriate fungus. Levels of root colonization by *F. oxysporum*, all isolates of *Fusarium*, *Trichoderma* and *Cylindrocarpon*, or no fungi among the five treatments were statistically compared (P=0.05) using Tukey's HSD.

RESULTS AND DISCUSSION

Effects of the five pre-plant soil treatments on ponderosa and lodgepole pine seedling root infection are summarized in tables 1 and 2, respectively. For ponderosa pine, the only noticeable reduction in *Fusarium* root infection occurred in the MBC treatment; most sampled seedlings from the other treatments were infected with these fungi. All sampled lodgepole pine seedlings, regardless of treatment, were infected with *Fusarium* spp. (table 2).

Treatment effects on level of root colonization are summarized for ponderosa and lodgepole pine seedlings in tables 3 and 4, respectively. The lowest level of

Fusarium root colonization for ponderosa pine seedlings occurred after the MBC treatment (table 3); the highest level of root colonization occurred following both the mushroom compost and bare fallow with cultivation treatments. Significantly (P=0.05) less root colonization occurred following both the bare fallow without cultivation and sawdust amendment treatments. For lodgepole pine seedlings, significantly lower levels of *Fusarium* root colonization occurred after the MBC and bare fallow without cultivation treatments (table 4).

Cylindrocarpon root colonization of ponderosa pine seedling roots was low for all treatments (table 3). However, relatively high levels of root colonization by *Cylindrocarpon* spp. were found on lodgepole pine seedlings from the two bare fallow treatments (table 4). *Cylindrocarpon* spp. are common rhizosphere inhabitants that often colonize root cortical cells (Booth 1966; James et al. 1994a). They may be pathogenic under certain conditions (Booth 1966; Dumroese et al. 2000; James et al. 1994a), but are routinely saprophytic or not very aggressive pathogens.

Trichoderma spp. was assayed on seedling roots at the highest levels on both conifer species following the MBC treatment. Several species of *Trichoderma* are considered potential biological control agents for controlling root diseases (Papavizas 1985). They may be competitive with or antagonistic toward soil borne pathogens. *Trichoderma* spp. often initially colonizes soil fumigated with general biocides (James 1989; Papavizas 1985) and they rapidly establish relatively high populations in such soils.

Four morphologically distinct groups (morphotypes) of *F. oxysporum* Schlecht. isolates were obtained from the roots of ponderosa pine seedlings (tables 1 and 3); three of these same morphotypes were found

Table 1. Effects of pre-plant soil treatments on infection of 1-0 bareroot ponderosa pine seedlings by selected fungi – USDA Forest Service Lucky Peak Nursery, Boise, Idaho[1].

Fungus[2]	Treatment[3]				
	A	B	C	D	E
FOXY 1[4]	100	85	95	90	35
FOXY 2	10	35	0	35	15
FOXY 3	30	10	0	0	0
FOXY 4	5	0	0	0	0
ALL FOXY	100	95	95	90	45
FPRO	0	0	60	10	15
FSAM	0	10	0	5	0
FACU	0	0	35	20	0
ALL FUS	100	95	100	95	60
TRICHO	90	100	80	65	100
CYLINDRO	0	25	0	0	0

[1] Values in table are percent of sampled seedlings (20 per treatment) infected with appropriate fungus.
[2] Abbreviations: FOXY = *Fusarium oxysporum*; FPRO = *F. proliferatum*; FSAM = *F. sambucinum*; FACU = *F. acuminatum*; FUS = *Fusarium*: TRICHO = *Trichoderma* spp.; CYLINDRO = *Cylindrocarpon* spp.
[3] Treatments: A = bare fallow with periodic cultivation; B = bare fallow without cultivation; C = mushroom compost amendment; D = sawdust amendment; E = methyl bromide/chloropicrin fumigation.
[4] *Fusarium oxysporum* isolates designated 1-4 based on different morphotypes on potato dextrose agar.

on lodgepole pine seedling roots (tables 2 and 4). All these isolates had morphologies characteristic of *F. oxysporum* (chlamydospores, microconidia produced on short, unbranched monophialides, characteristic macroconidia) (James et al. 1989a, 1991; Nelson et al. 1983). Morphotypes were primarily differentiated based on abundance of aerial mycelium, pionnotes and sporodochia, and levels of violet pigmentation. These differences may not necessarily be related to level of virulence on seedling roots (Gordon and Martyn 1997; James et al. 1989b, 2000). Experience indicates that pathogenic and saprophytic isolates of *F. oxysporum* cannot be separated morphologically (Bloomberg 1976; Gordon and Martyn 1997; Gordon and Okamoto 1992a; James et al. 2000). However, molecular techniques have recently been used to differentiate *F. oxysporum* populations based on host range, level of virulence, and saprophytic ability (Appel and Gordon 1995; Gordon and Okamoto 1992a, 1992b, 1992c).

Since all the seedlings sampled in this evaluation appeared healthy, it was suspected that the majority of the *F. oxysporum* isolates colonizing roots were probably saprophytic or only weakly pathogenic (Bloomberg and Lock 1972; James and Gilligan 1988a, 1988b). Previous work indicated that *F. oxysporum* readily colonizes root cortical tissues at relatively high levels without eliciting tissue necrosis or initiating disease symptoms (James and Gilligan 1988a, 1988b). In some cases when seedlings become stressed, *F. oxysporum* may become pathogenic, resulting in typical root disease (James et al. 1991). The possibility and level of disease may be related to extent of root colonization as well as the proportion of the *Fusarium* population comprised of virulent isolates (Bloomberg and Lock 1972; Gordon and Martyn 1997; James et al. 1989b, 2000). Therefore, one major disease management goal should be to reduce root infection by *Fusarium* spp. as much as possible (James et al. 1991).

Table 2. Effects of pre-plant soil treatments on infection of 1-0 bareroot lodgepole pine seedlings by selected fungi – USDA Forest Service Lucky Peak Nursery, Boise, Idaho[1].

Fungus[2]	Treatment[3]				
	A	B	C	D	E
FOXY 1[4]	100	90	100	100	100
FOXY 2	10	5	90	0	0
FOXY 3	0	50	0	0	0
ALL FOXY	100	100	100	100	100
FPRO	5	0	0	20	0
FSAM	30	0	0	5	5
FACU	0	0	0	0	5
FSOL	0	0	20	0	0
ALL FUS	100	100	100	100	100
TRICHO	85	95	45	45	95
CYLINDRO	90	65	5	15	0

[1] Values in table are percent of sampled seedlings (20 per treatment) infected with appropriate fungus.
[2] Abbreviations: FOXY = *Fusarium oxysporum*; FPRO = *F. proliferatum*; FSAM = *F. sambucinum*; FACU = *F. acuminatum*; FSOL = *F. solani*; FUS = *Fusarium*: TRICHO = *Trichoderma* spp.; CYLINDRO = *Cylindrocarpon* spp.
[3] Treatments: A = bare fallow with periodic cultivation; B = bare fallow without cultivation; C = mushroom compost amendment; D = sawdust amendment; E = methyl bromide/chloropicrin fumigation.
[4] *Fusarium oxysporum* isolates designated 1-3 based on different morphotypes on potato dextrose agar.

Table 3. Effects of pre-plant soil treatments on root colonization of 1-0 bareroot ponderosa pine seedlings by selected fungi – USDA Forest Service Lucky Peak Nursery, Boise, Idaho[1].

Fungus[2]	Treatments[3]				
	A	B	C	D	E
FOXY 1[4]	44.5	21.0	41.5	26.0	6.5
FOXY 2	1.5	5.0	0	4.0	1.5
FOXY 3	4.0	1.5	0	0	0
FOXY 4	1.5	0	0	0	0
ALL FOXY	51.0 A	27.0 B	41.5 AB	29.0 B	8.0 C
FPRO	0	0	16.5	1.0	2.5
FSAM	0	1.5	0	0.5	0
FACU	0	0	7.0	3.5	0
ALL FUS	51.0 A	28.0 B	63.0 A	34.0 B	10.5 C
TRICHO	41.0 BC	61.5 B	29.0 C	29.0 C	90.0 A
CYLINDRO	0 B	6.0 A	0 B	0 B	0 B
NONE	25.5 B	15.0 BC	17.5 BC	45.0 A	7.5 C

[1] Values in table are percent of root pieces (10 sampled per seedling; 20 seedlings sampled per treatment) colonized with appropriate fungus. Within particular rows, means followed by the same capital letter are not significantly different (P=0.05) using Tukey's HSD.
[2] Abbreviations: FOXY = *Fusarium oxysporum*; FPRO = *F. proliferatum*; FSAM = *F. sambucinum*; FACU = *F. acuminatum*; FUS = *Fusarium*; TRICHO = *Trichoderma* spp.; CYLINDRO = *Cylindrocarpon* spp.; NONE = no fungi (may or may not have been colonized by bacteria).
[3] Treatments: A = bare fallow with periodic cultivation; B = bare fallow without cultivation; C = mushroom compost amendment; D = sawdust amendment; E = methyl bromide/chloropicrin fumigation
[4] *Fusarium oxysporum* isolates designated 1-4 based on different morphotypes on potato dextrose agar.

Other *Fusarium* spp. isolated from seedling roots included *F. proliferatum* (Matsushima) Nirenberg, *F. sambucinum* Fuckel, *F. acuminatum* Ell. & Ev., and *F. solani* (Mart.) Appel. & Wollenw. All these species were found on roots at much lower levels than *F. oxysporum*. Most of these other *Fusarium* isolates were probably saprophytic, although tests have indicated that certain isolates of *F. proliferatum* (James et al. 1997), *F. acuminatum* (James 2000) and *F. solani* (James and Perez 2000) may be pathogenic on conifer seedlings.

Seedling density after two growing seasons (at lifting) is probably the best measure of soil treatment effects (Stone et al. 1997). Seedling height and diameter may be related to level of pathogen activity on roots but may also reflect differences in seedling density. Generally, treatment effects on seedling density coincided with level of *Fusarium* root infection at the Lucky Peak Nursery. MBC treatments produced denser stands of ponderosa and lodgepole pine seedlings as well as lower levels of root colonization by *Fusarium* spp. Fallowing without cultivation also produced denser lodgepole pine seedlings and lower levels of root colonization by potential pathogens. Fallowing had similar effects on ponderosa pine seedlings. However, experience at the nursery has indicated that fallowing alone cannot reduce soil *Fusarium* populations sufficiently. Higher than acceptable disease levels still occur (James and Beall 2000).

Table 4. Effects of pre-plant soil treatments on root colonization of 1-0 bareroot lodgepole pine seedlings by selected fungi – USDA Forest Service Lucky Peak Nursery, Boise, Idaho[1].

Fungus[2]	Treatments[3]				
	A	B	C	D	E
FOXY 1[4]	73.0	33.5	96.5	73.5	44.0
FOXY 2	1.0	0.5	16.0	0	0
FOXY 3	0	12.5	0	0	0
ALL FOXY	73.0 A	46.0 B	97.5 A	73.5 A	44.0 B
FPRO	1.0	0	0	3.0	0
FSAM	3.0	0	0	1.0	1.0
FACU	0	0	0	0	0.5
FSOL	0	0	2.0	0	0
ALL FUS	73.0 A	46.0 B	98.5 A	76.0 A	45.5 B
TRICHO	20.0 BC	36.5 AB	4.5 D	9.5 CD	53.0 A
CYLINDRO	30.0 A	23.0 A	0.5 B	1.5 B	0 B
NONE	8.5 B	13.5 AB	1.0 C	18.5 A	17.5 A

[1] Values in table are percent of root pieces (10 sampled per seedling; 20 seedlings sampled per treatment) colonized with appropriate fungus. Within particular rows, means followed by the same capital letter are not significantly different (P=0.05) using Tukey's HSD.

[2] Abbreviations: FOXY = *Fusarium oxysporum*; FPRO = *F. proliferatum*; FSAM = *F. sambucinum*; FACU = *F. acuminatum*; FSOL = *F. solani*; FUS = *Fusarium*; TRICHO = *Trichoderma* spp.; CYLINDRO = *Cylindrocarpon* spp.; NONE = no fungi (may or may not have been colonized by bacteria).

[3] Treatments: A = bare fallow with periodic cultivation; B = bare fallow without cultivation; C = mushroom compost amendment; D = sawdust amendment; E = methyl bromide/chloropicrin fumigation

[4] *Fusarium oxysporum* isolates designated 1-3 based on different morphotypes on potato dextrose agar.

Assaying healthy-appearing seedlings for *Fusarium* root infection may be an easy, indirect way of determining disease potential. In the past, disease potential has most often been estimated from overall *Fusarium* populations within soil (James et al. 1991, 1994b, 1996; Stone et al. 1997). However, isolates that infect seedling roots are of most concern since they are the ones that can potentially initiate disease. Levels of root colonization by *Fusarium* may reflect greater potential hazard to seedlings than numbers of propagules residing within nursery soils. Fortunately, most *Fusarium* colonizing roots of healthy seedlings is replaced by other mycoflora (particularly mycorrhizal symbionts) once seedlings are outplanted on forest sites (Dumroese et al. 1993). Seedlings leaving nurseries with low to moderate levels of *Fusarium* root colonization usually perform well after outplanting (Dumroese et al 1993; Smith 1967).

Continued efforts are underway to develop satisfactory alternatives to pre-plant soil fumigation with MBC at the Lucky Peak Nursery. Fallowing and the alternative chemical fumigant dazomet are currently not viable alternatives (James and Beall 1999, 2000). Supplementing fallowing or alternative chemicals with biocontrol agents may help. In addition, other chemicals (methyl iodide; chloropicrin alone) warrant investigation. It is unlikely that any alternative will be as effective as methyl bromide/chloropicrin fumigation has been in the past.

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R.L. James is Plant Pathologist, USDA Forest Service, Northern Region, Forest Health Protection. Address: USDA Forest Service, 3815 Schreiber Way, Coeur d'Alene, ID 83814; email: rjames@fs.fed.us